Use of Biologic Diagnostics for Precision Oral Health: What Role in the Public Health Sector?

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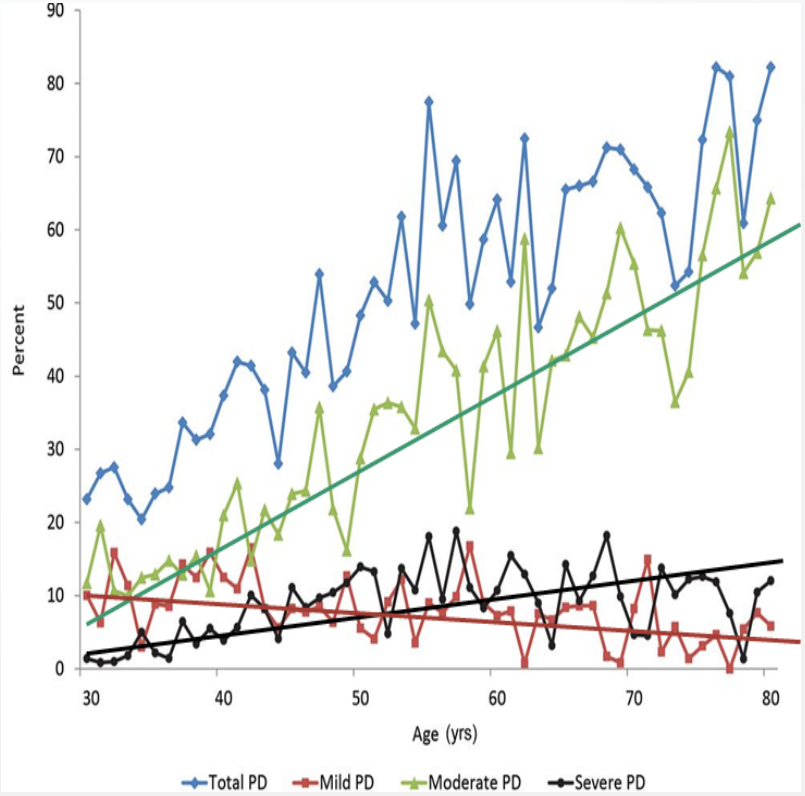
Periodontitis: Basic Concepts

- Infection with specific bacteria or pathogenic consortia in biofilms
  - Different complexes in health and disease
  - Pathogens (opportunistic commensals)
  - Triggered by changes in the oral microbiome with some specific bacteria helping to create a "dysbiosis" and pathogenic biofilm
- Host immunoinflammatory response (local & systemic)
  - Biofilms "trigger" a chronic inflammatory response that destroys soft tissues (*ie.* gingiva) & hard tissue (*ie.* alveolar bone)
  - Dysregulation of the homeostasis of host responses
- "High Risk" population (pathogens; genetic regulation of host response)

"Support bacteria - they're the only culture some people have." – Steven Wright

Periodontal Disease: Epidemiologic Concepts

(NHANES 2009-2012)



P.I. Eke et al. J DENT RES 2012;91:914-920

	Name	Percentage of Periodontitis	Name	Percentage of Periodontitis	
1	Male	13.8%	Female	8.2%	<.0001
2	Mexican American	14.3%	Other Hispanic	14.9%	.3817
3	Mexican American	14.3%	NonHispanic White	6.4%	<.0001
4	Mexican American	14.3%	NonHispanic Black	17.2%	.0086
5	Mexican American	14.3%	Other race	11.3%	.0734
6	Other Hispanic	14.9%	NonHispanic White	6.4%	<.0001
7	Other Hispanic	14.9%	NonHispanic Black	17.2%	.1322
8	Other Hispanic	14.9%	Other race	11.3%	.0807
9	NonHispanic White	6.4%	NonHispanic Black	17.2%	<.0001
10	NonHispanic White	6.4%	Other race	11.3%	.0003
11	NonHispanic Black	17.2%	Other race	11.3%	.0047
12	Age 18~30	4.3%	Age 31~49	12.9%	<.0001
13	Age 18~30	4.3%	Age 50~64	13.9%	<.0001
14	Age 18~30	4.3%	Age 65+	14.7%	<.0001
15	Age 31~49	12.9%	Age 50~64	13.9%	.1646
16	Age 31~49	12.9%	Age 65+	14.7%	.0625
17	Age 50~64	13.9%	Age 65+	14.7%	.2979

JDR JOURNAL OF DENTAL RESEARCH

"Hidden Costs" of Oral Infections Across the Life Span

- Children
  - Normal Growth & Development
    - Nutrition
    - Sleep
    - Pain
    - Ability ot Learn
    - Missed School Days
    - Self-image/Appearance

- Employees
  - High Out-of-Pocket Costs
  - Lost Work Days
  - Caregiving
  - Adverse Birth Outcomes
  - Presenteeism - ↓Productivity
    - Impact on general health

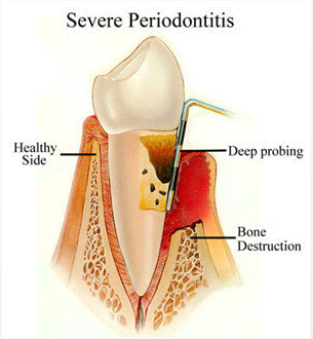
"Hidden Costs" of Oral Infections Across the Life Span

- Employers
  - Direct costs of dental benefits/ treatment
  - Absenteeism
  - Presenteeism - ↓Productivity
  - Retiree health care costs

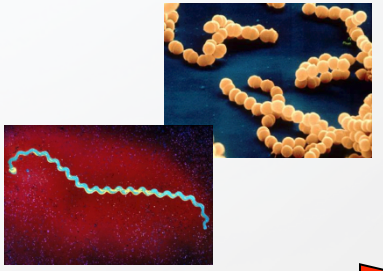
- Senior Citizens/Retirees
  - High Out-of-Pocket costs
  - Lack of dental insurance
    - No outpatient Medicare benefits
  - Impact on general health
    - Lost years of quality of life and lifespan

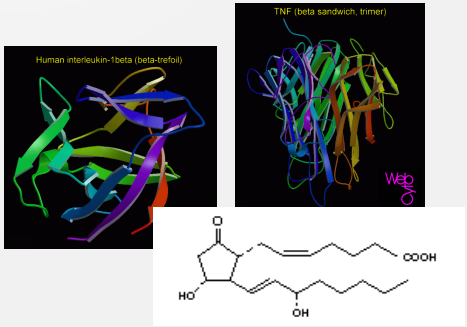
Oral-Systemic Disease

Altered Microbiota
Increased Pathogenicity
Altered Inflammation



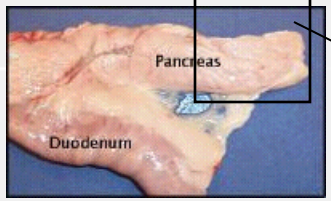
Translocated Infection

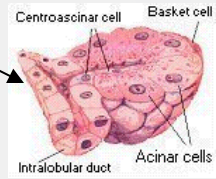




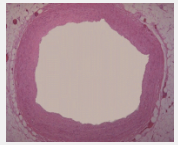
Systemic Inflammatory Mediators

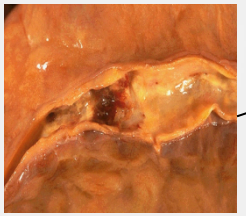
Diabetes

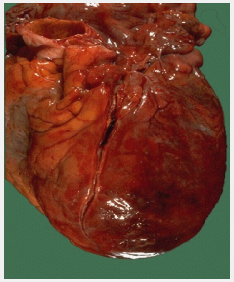




CVD





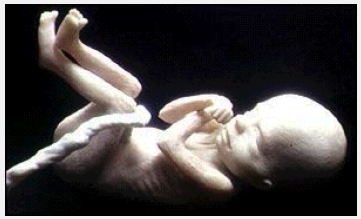


Endothelium & altered vascular responses

Atherosclerotic changes

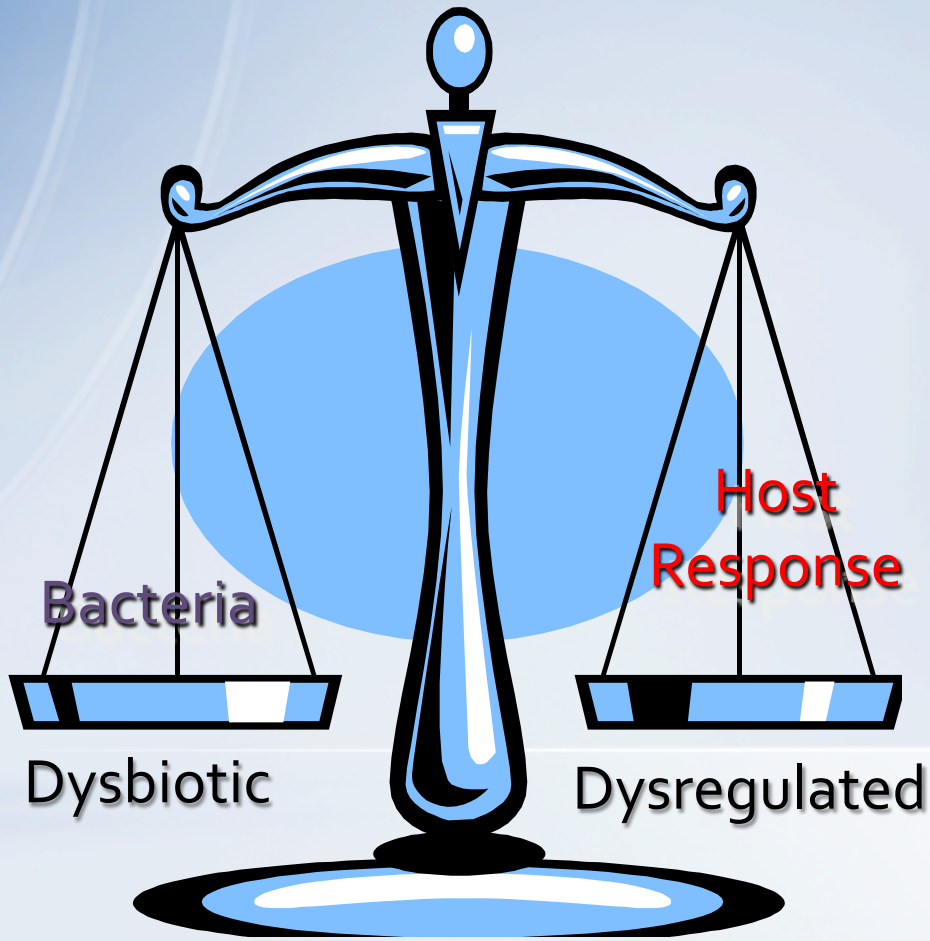
Coronary Artery Disease (MI)

PTB/IUGR

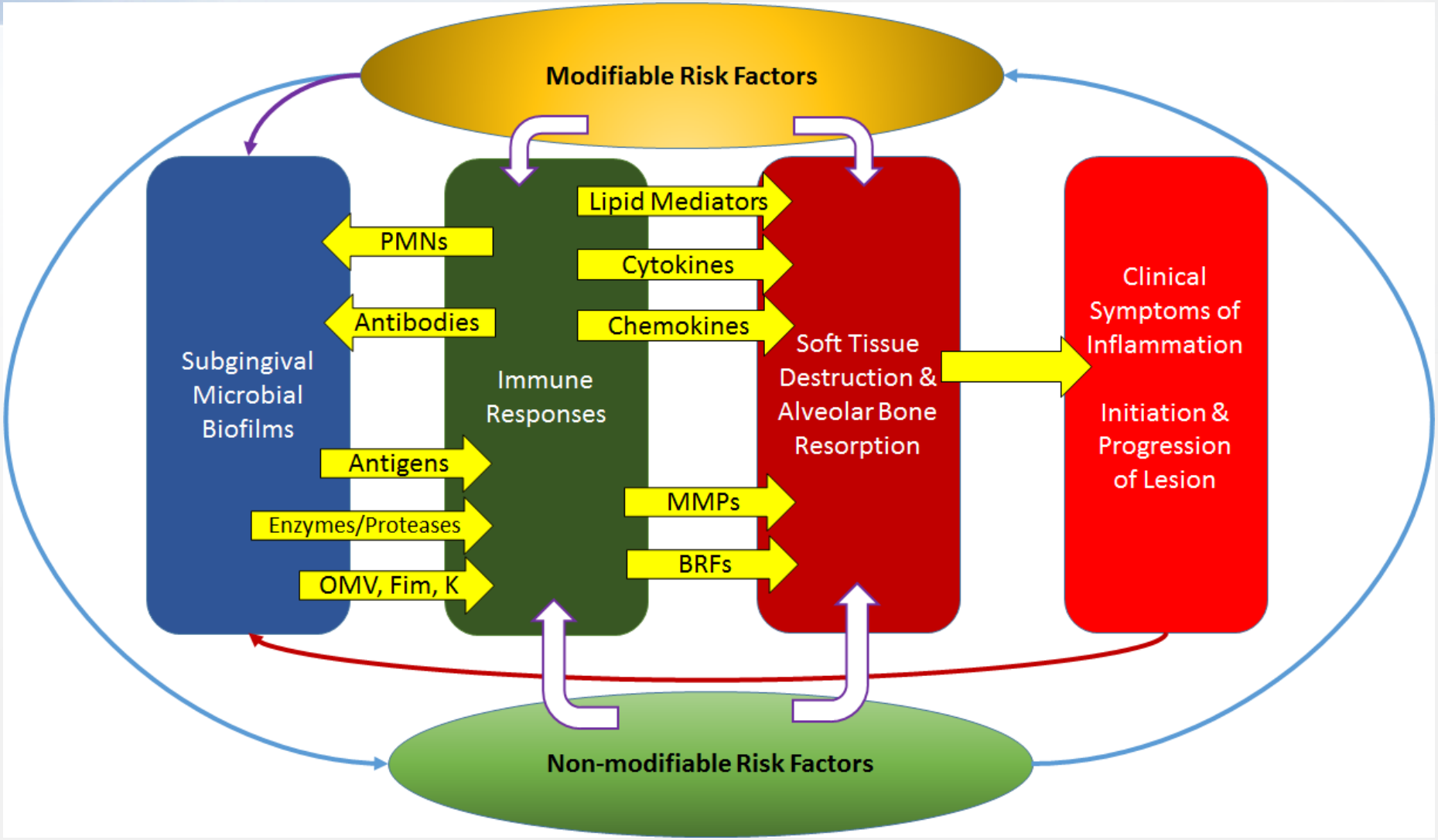


Alzheimer's Disease

PERIODONTAL DISEASE: THE DUAL CHALLENGE



Host Responses & Periodontitis

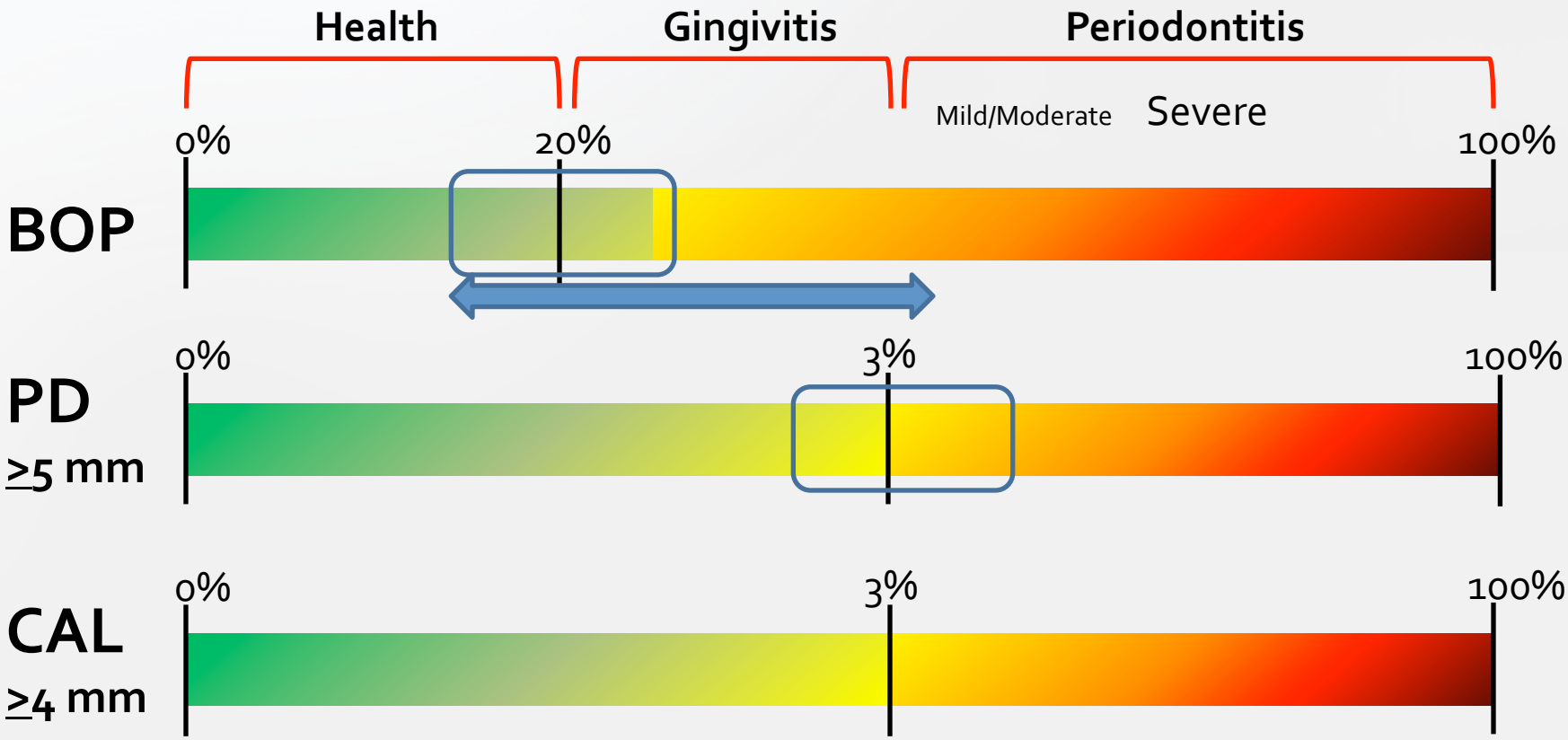


Modified from Kornman

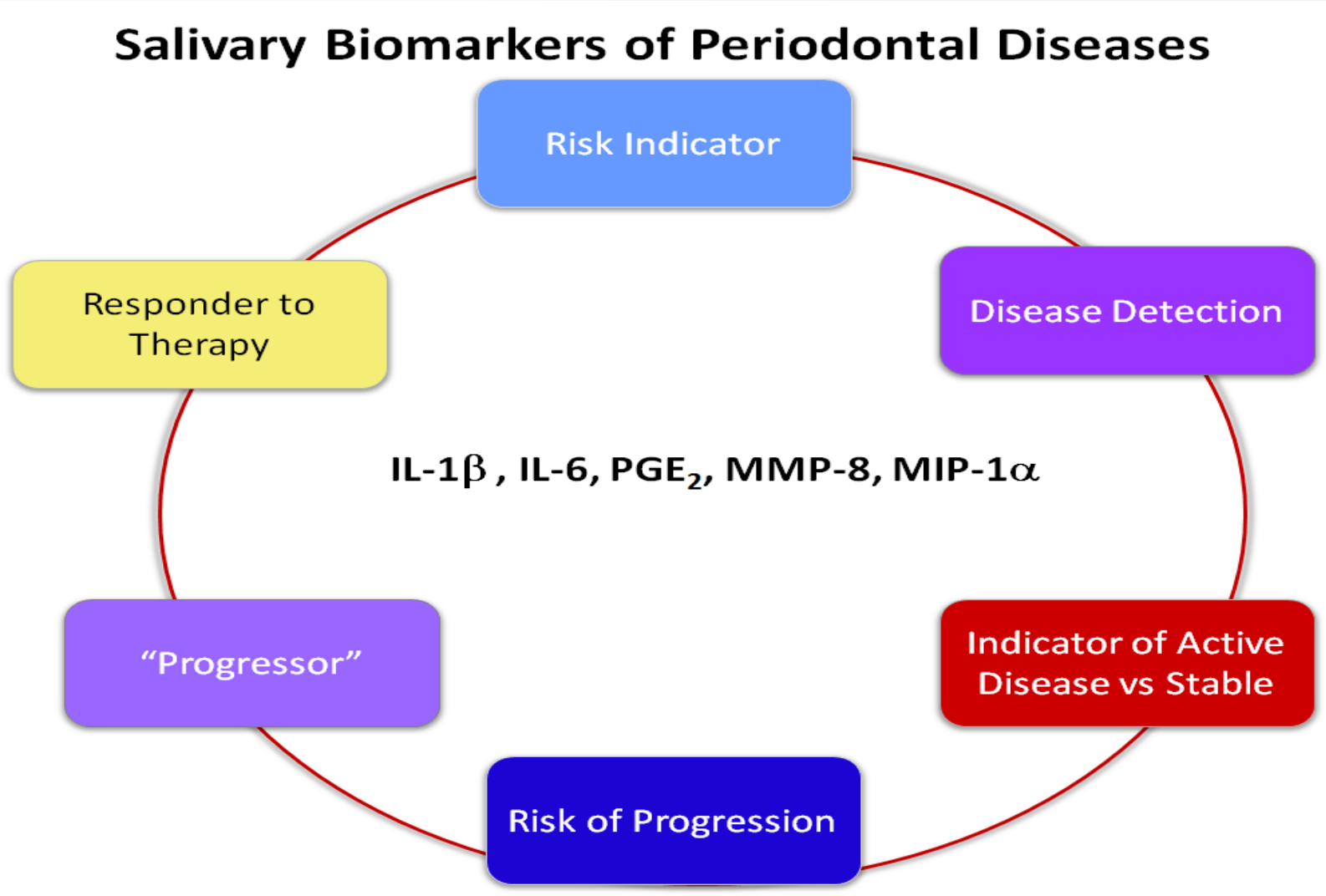
Biologic Diagnostics & Periodontitis

"The Challenge"

Clinical Evaluation and Categorization of Health, Gingivitis, and Periodontitis



Biomarkers in Periodontal Diseases



Gingivitis & Salivary Analytes

?

?

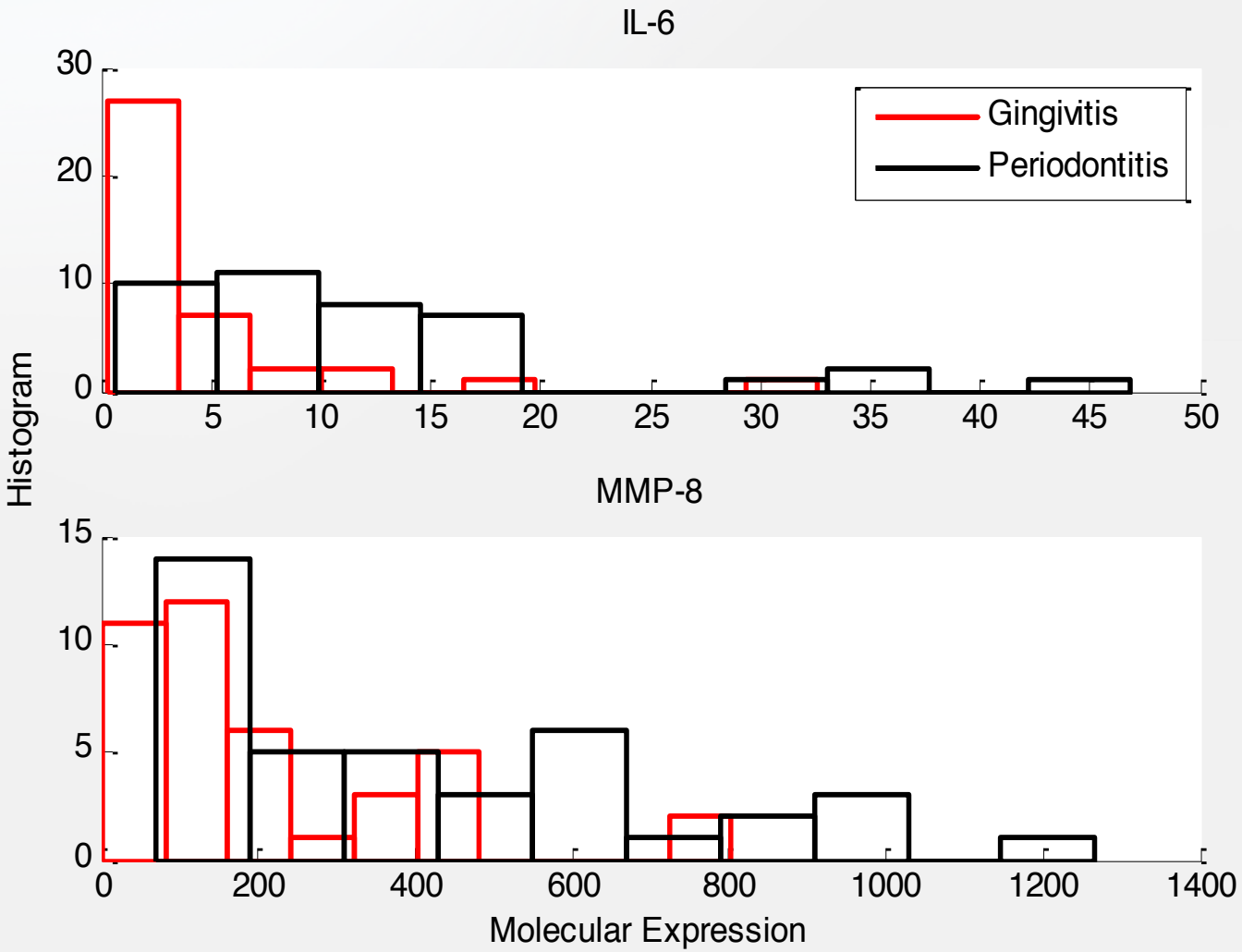
Comparisons of concentrations of biomarkers in healthy and gingivitis groups and post-treatment of gingivitis subjects

Biomarkers	Avg H1-H2 vs Avg G1-G2
IL-1b	0.258
IL-6	0.435
MMP-8	0.327
MIP-1a	0.002
PGE_2	0.001

Analytes	H vs Post-Tx (G3) P value
IL-1b	0.302
IL-6	0.765
MMP-8	0.966
MIP-1a	0.014
PGE_2	0.037

Concentrations are mean ± SD. P values by Wilcoxon signed rank (within groups) and rank sum (between groups) statistics.

Distribution of Salivary Analytes in Gingivitis & Periodontitis



Summary

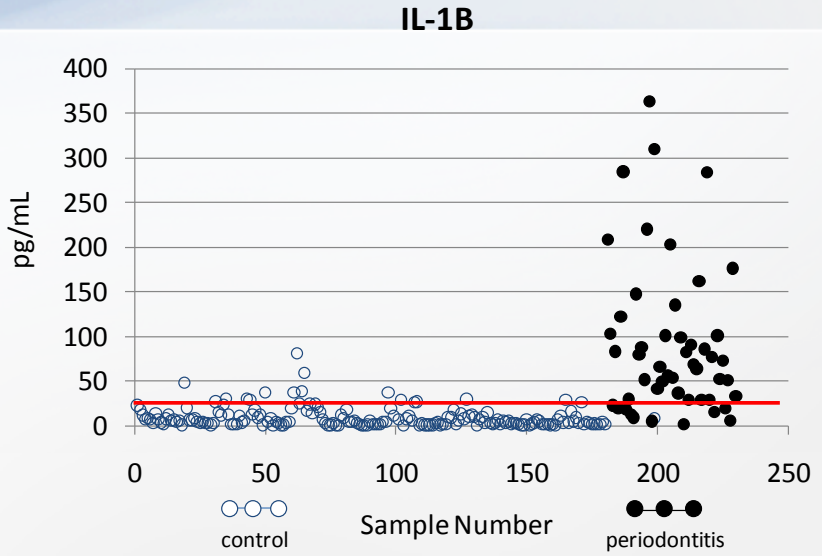
- Biologically gingivitis is different than periodontitis with respect to salivary biomarkers
- There is heterogeneity in the gingivitis patients noted biologically; albeit clinically grouped the same
- Subset of gingivitis patients demonstrate salivary biomarkers that are different and appear more like periodontitis
- What is the clinical future of this subset?

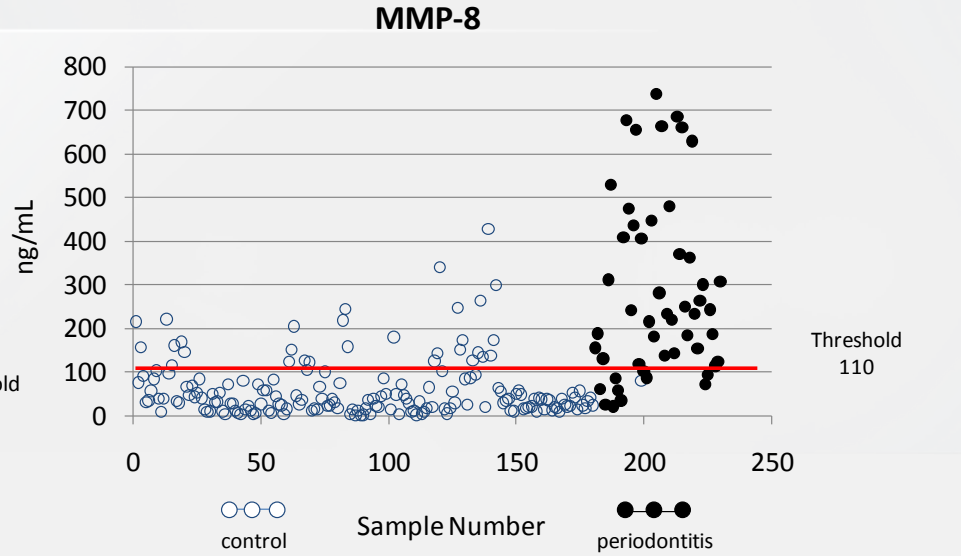
Comparisons of salivary analytes in health and disease

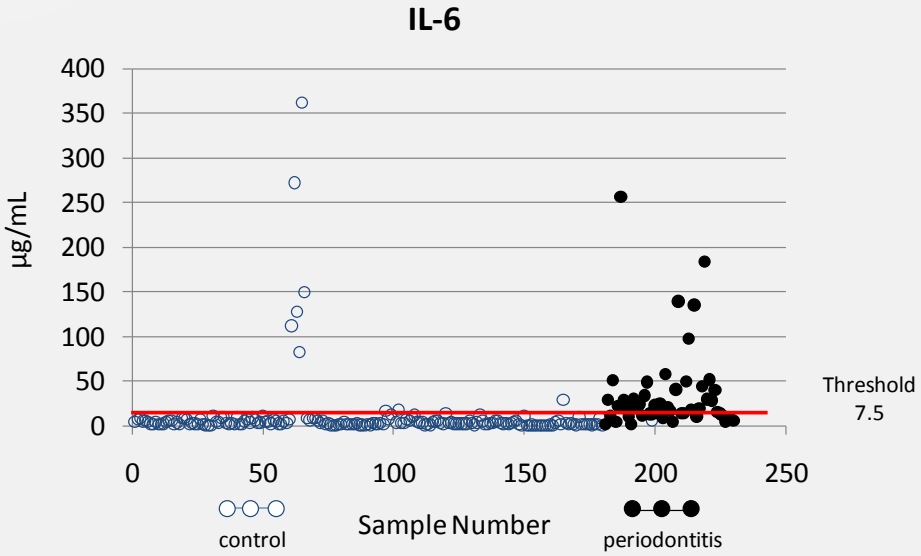
T-test	Healthy		Periodontal disease		p_value
	Mean	Std	Mean	Std	
IL-1β	7.24	7.69	90.94	85.22	<.0001
IL-6	3.30*	2.32	35.57	48.17	0.0006
INFα	27.65	48.29	3.19	2.29	0.0006
MMP-8	52.63	40.62	283.47	203.47	<.0001
PGE_2	179.95	155.31	226.07	314.69	0.4563
TNFa	1.85	2.11	5.44†	10.88	0.0800
Albumin	36.15	26.29	112.94	126.53	0.0016

* Denotes 29 samples tested; † Denotes 38 samples tested

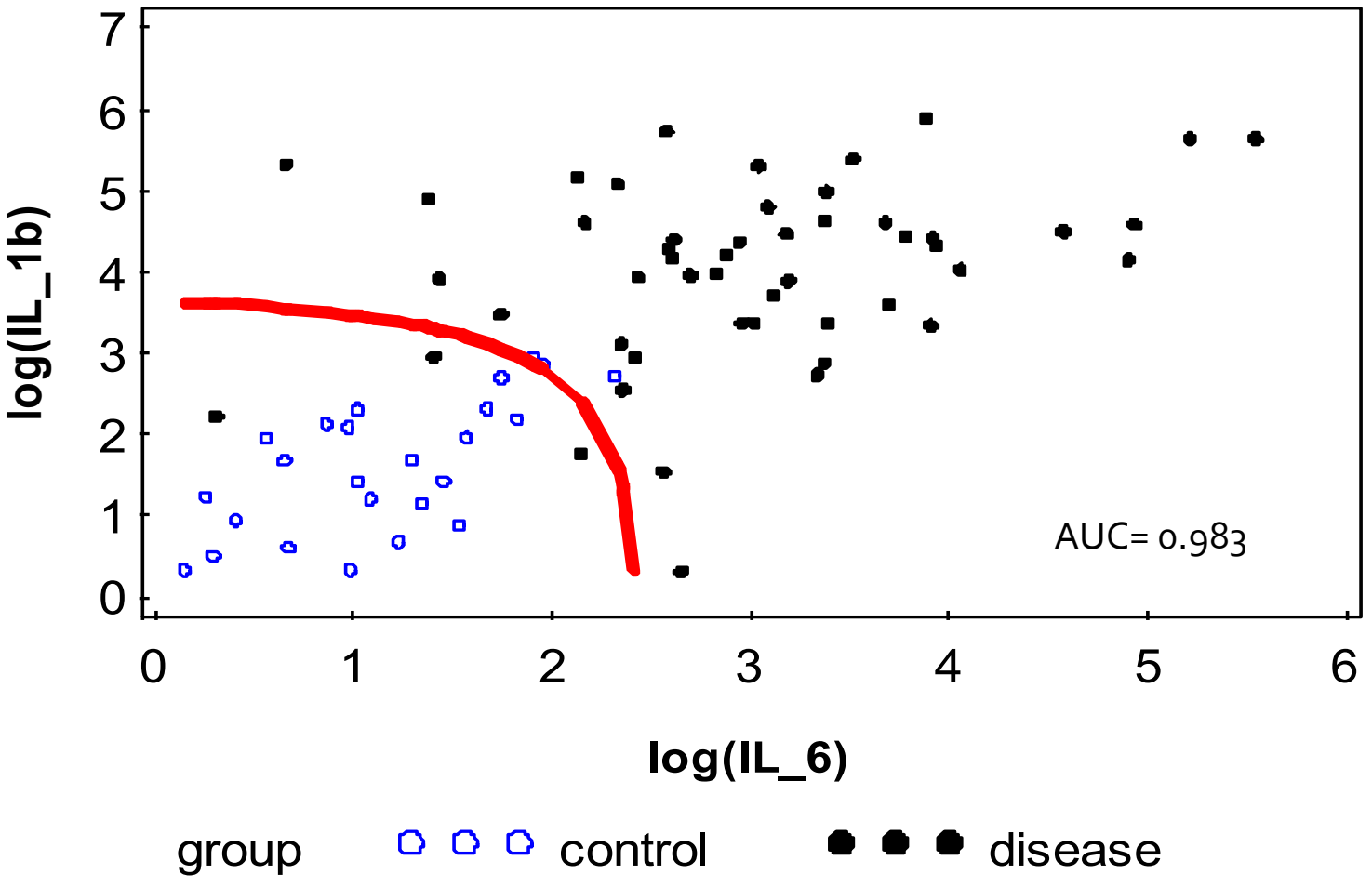
Within Subject: Salivary Analytes in Health & Periodontitis



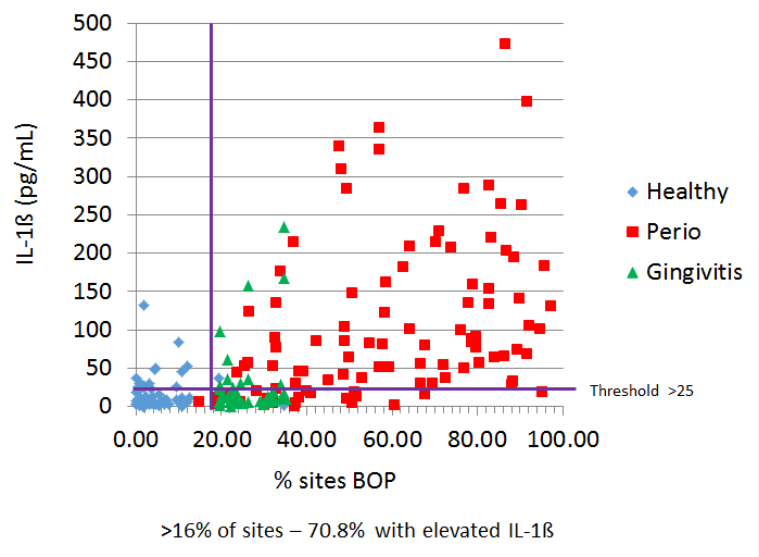




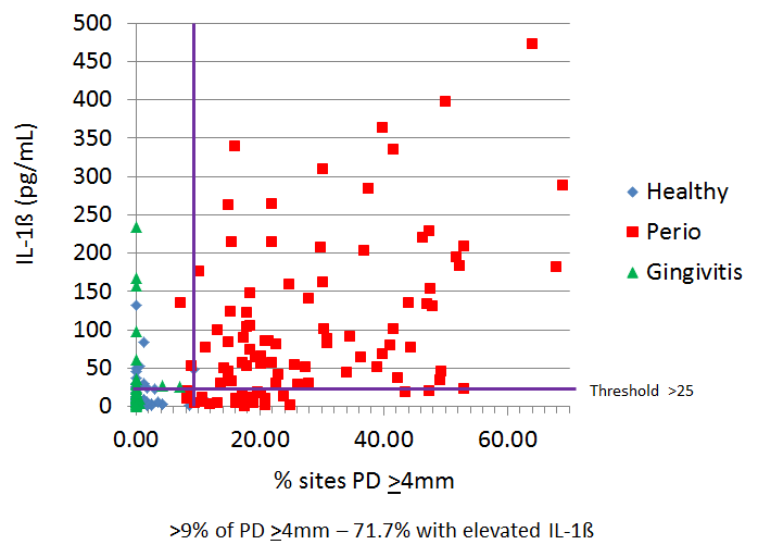
Biomarker Combinations



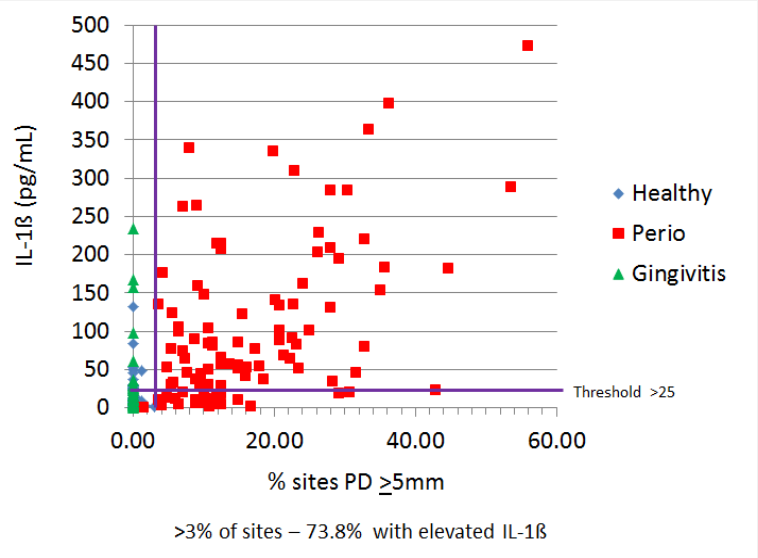
PD Extent/Severity and Salivary Analytes



>16% of sites – 70.8% with elevated IL-1ß

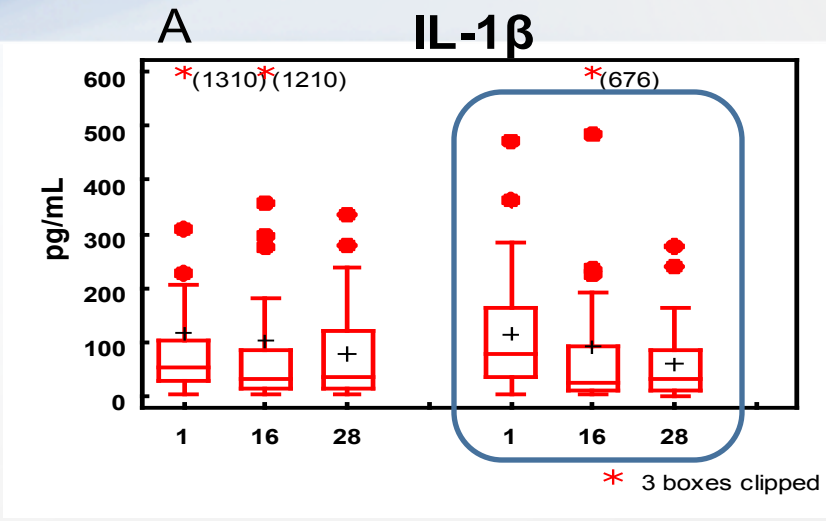


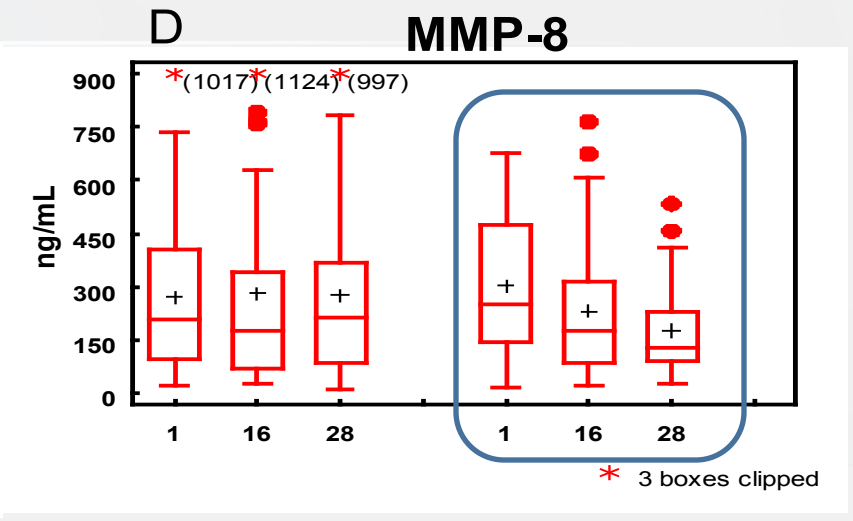
>9% of PD ≥4mm – 71.7% with elevated IL-1ß

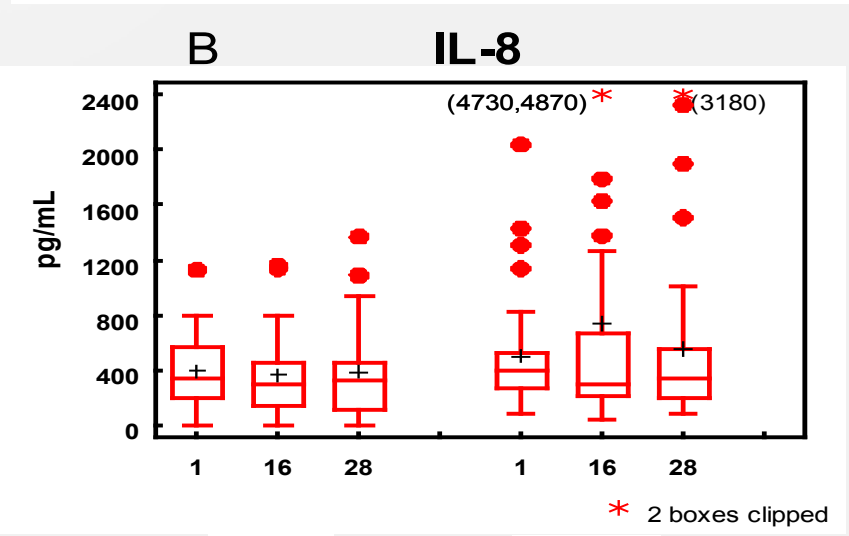


>3% of sites – 73.8% with elevated IL-1ß

Tx Effects on Salivary Biomarkers





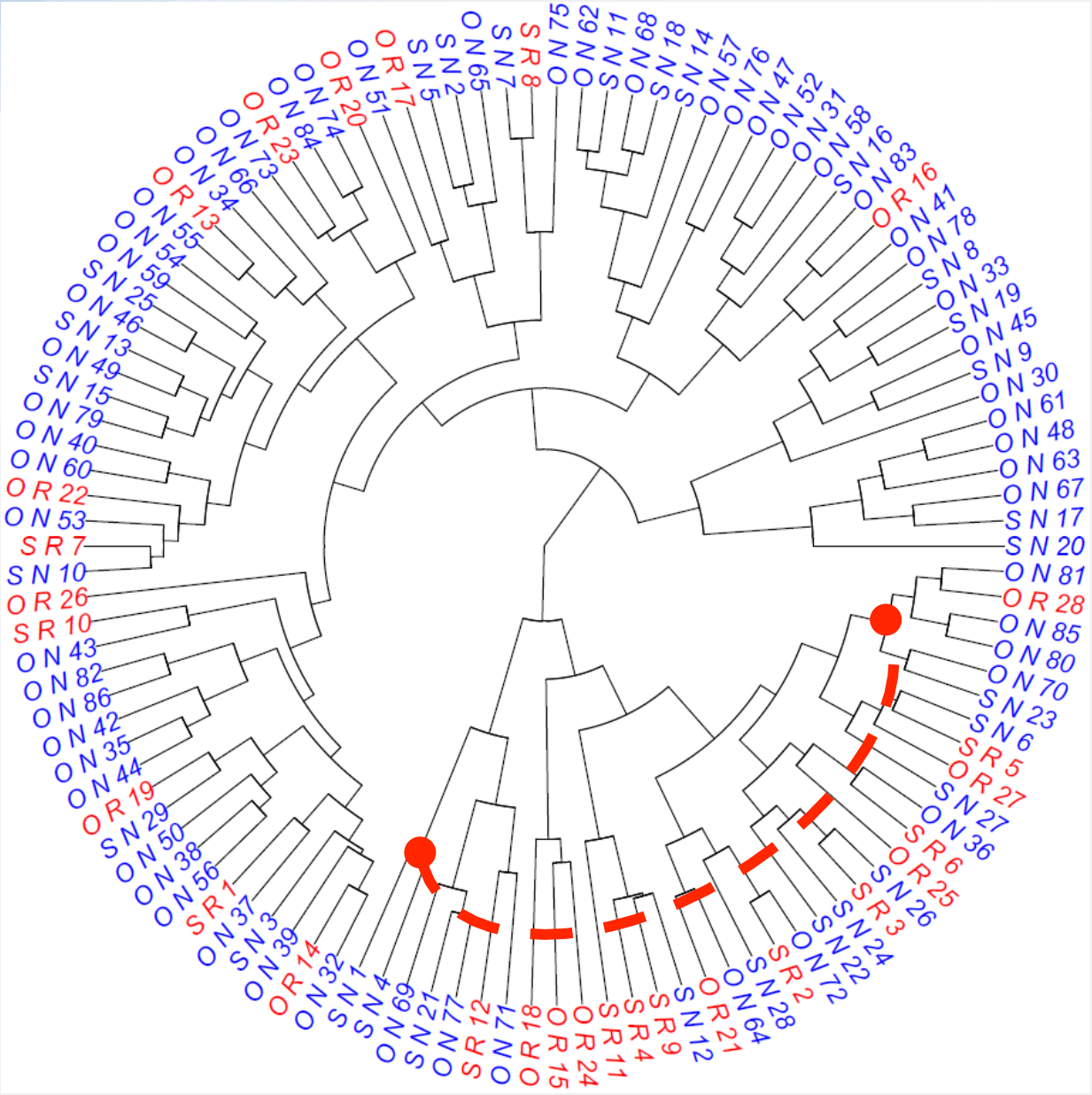


OHI

S/RP

Biomarkers used for Identifying Recurrent Attachment Loss (RAL) patients

Type	Symbol	Description
Serum	**ISO**	Isoprostane
Serum	**MMP1**	Matrix Metalloproteinase 1
Serum	**MMP2**	Matrix Metalloproteinase 2
Serum	**MMP9**	Matrix Metalloproteinase 9
Serum	**BPI**	Bactericidal permeability-increasing protein
Serum	**LBP**	Lipopolysaccharide Binding Protein
Serum	**SAP**	Serum Amyloid P
Serum	**CRP**	C-Reactive Protein
Serum	**Aa**	Aggregatibacter actinomycetemcomitans
Serum	**Pg**	Porphyromonas gingivalis
Serum	**Td**	Treponema denticola
Serum	**Pi**	Prevotella intermedia
Serum	**Tf**	Tannerella forsythia
Serum	**Cr**	Campylobacter rectus
Serum	**Fn**	Fusobacterium nucleatum
Salivary	**PGE2**	Prostaglandin E2
Salivary	**IL1b**	Interleukin 1b
Salivary	**IL6**	Interleukin 6
Salivary	**INFa**	Interferon alpha
Salivary	**TNFa**	Tumor Necrosis Factor alpha
Salivary	**MMP8**	Matrix Metalloproteinase 8
Salivary	**ALB**	Albumin
Salivary	**CRP**	C-Reactive Protein
Plaque	**Pg**	Porphyromonas Gingivalis
Plaque	**Tf**	Tannerella forsythia
Plaque	**Td**	Treponema denticola
Plaque	**Tot**	Total bacteria


S R 8
O N 75
O N 29
S N 11
O N 69
S N 18
O N 14
O N 57
O N 76
O N 47
O N 52
O N 31
O N 58
S N 16
O N 83
O R 16
O N 41
O N 78
S N 8
O N 33
S N 19
O N 45
S N 9
O N 30
O N 61
O N 48
O N 63
O N 67
S N 17
S N 20
O N 81
O R 28
O N 85
O N 80
O N 70
S N 23
S N 6
S R 5
O R 21
S N 27
O N 36
O R 6
O R 25
S N 26
S R 3
S N 24
O N 22
O N 72
S R 2
S N 28
O N 64
O R 21
S N 12
S R 9
S R 4
S R 11
O R 24
O R 15
O R 18
O N 71
S R 12
O N 77
O N 21
S N 4
S N 1
O N 32
O R 14
O N 39
S N 3
O N 37
S R 1
O N 56
O N 38
O N 50
S N 29
O R 19
O N 44
O N 35
O N 42
O N 86
O N 82
O N 43
S R 10
O R 26
S N 10
S R 7
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O R 22
O N 60
O N 40
O N 79
S N 15
O N 49
S N 13
O N 46
S N 25
O N 59
O N 54
O N 55
O R 13
O N 34
O N 66
O N 73
O R 23
O N 74
O N 84
O R 20
O N 51
O R 17
S N 5
S N 2
O N 65
S N 7

Performance of different classification approaches in discerning RAL and Non-RAL subjects

Classification Approach	Sensitivity	Specificity
Traditional Single Classifier System		
NB	0.72±0.13	0.55±0.08
SVM	0.68±0.16	0.56±0.07
LDA	0.56±0.16	0.51±0.07
SVA Classification Framework		
SVA-NB	0.88±0.09	0.45±0.07
SVA-SVM	0.82±0.11	0.50±0.06
SVA-LDA	0.84±0.10	0.47±0.05

Conclusions: Periodontitis

- Selected biomarkers of host responses are indicative of periodontitis
- Subset of gingivitis patients show biomarker patterns consistent with periodontitis
- Targeted threshold levels of biomarkers are related to severity and extent of periodontal disease
- Combinations of biomarkers have high capacity to identify disease
- Some stability of biomarkers within individual subjects
- Biomarkers respond to periodontal therapy
- Biomarkers can predict the likelihood of progressing disease even after nonsurgical therapy

Periodontitis and Systemic Diseases

Diagnostics & AMI

Overall Goals

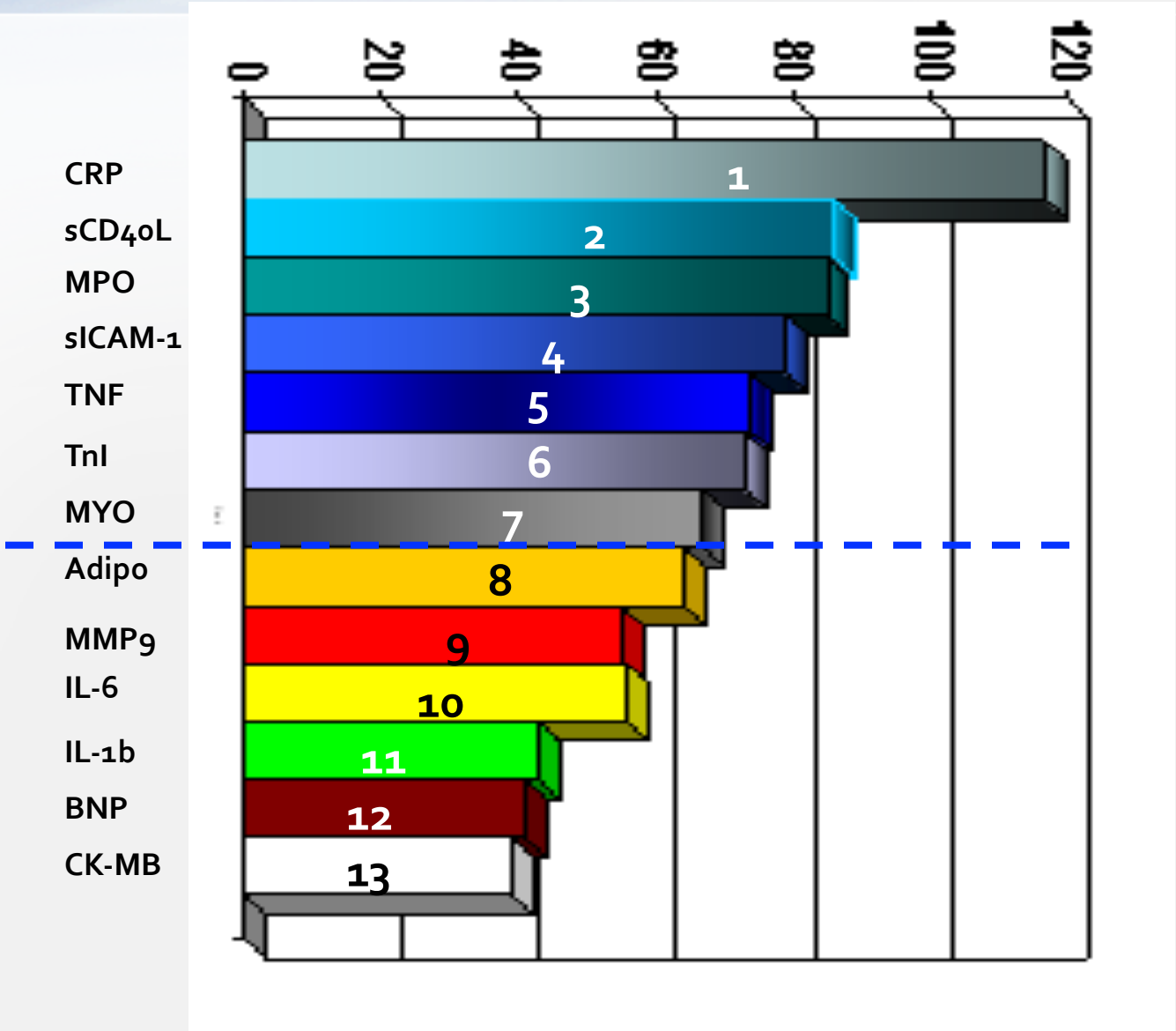
1. Oral fluids contain biomarkers of ACS that will contribute to strategy of earlier diagnosis.

2. Biomarker Discovery

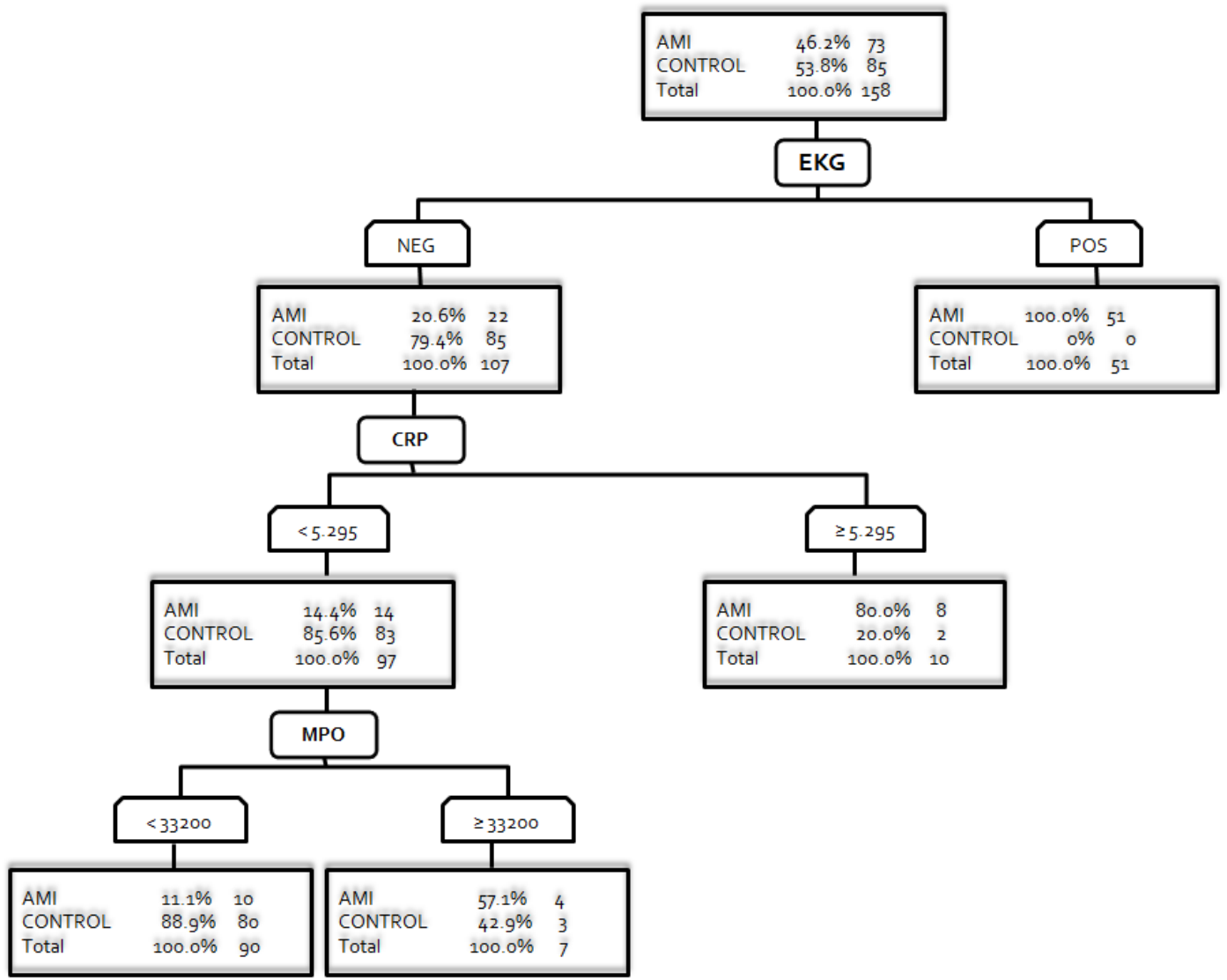
   A. Panel of cardiac biomarkers in oral fluids that distinguish AMI from health.

   B. Analyze kinetic pattern of salivary biomarkers in carefully monitored, chemically-induced MIs.

Algorithm: Weighted Analyses
Final Biomarker Ranking for Salivary Analytes in AMI



CART Analysis of Salivary Biomarkers in AMI



Conclusions

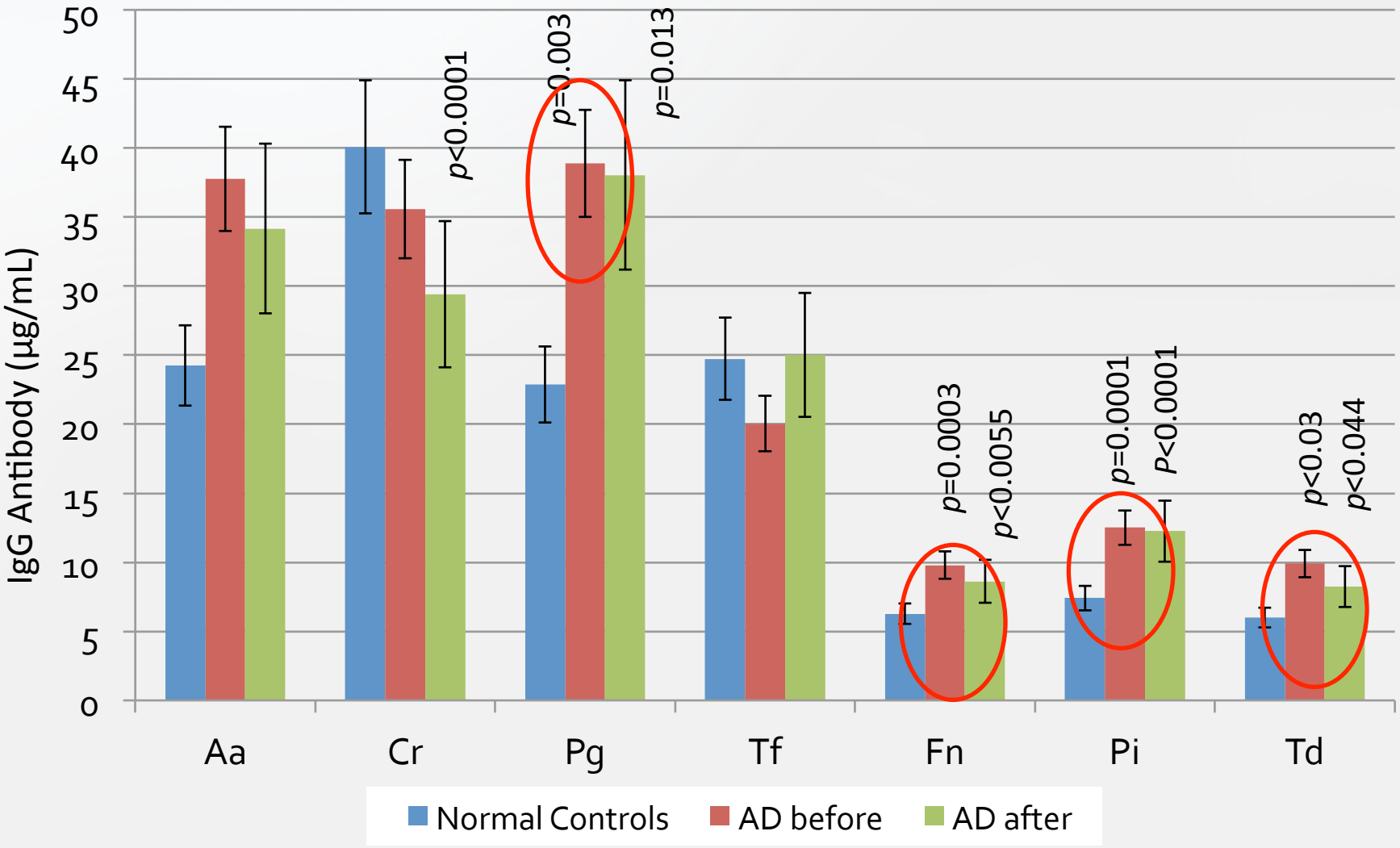
- UWS biomarker panel (CRP, sCD40L, MPO, sICAM-1, TnI, MYO) has utility for distinguishing AMI from healthy controls

- Logistic regression (AUC) and CART show that UWS panels yield sensitivity >80% and specificity up to 93% for AMI diagnosis

- Kinetic and ablation analysis demonstrated select UWS biomarkers appear during specific "windows" of heart tissue damage

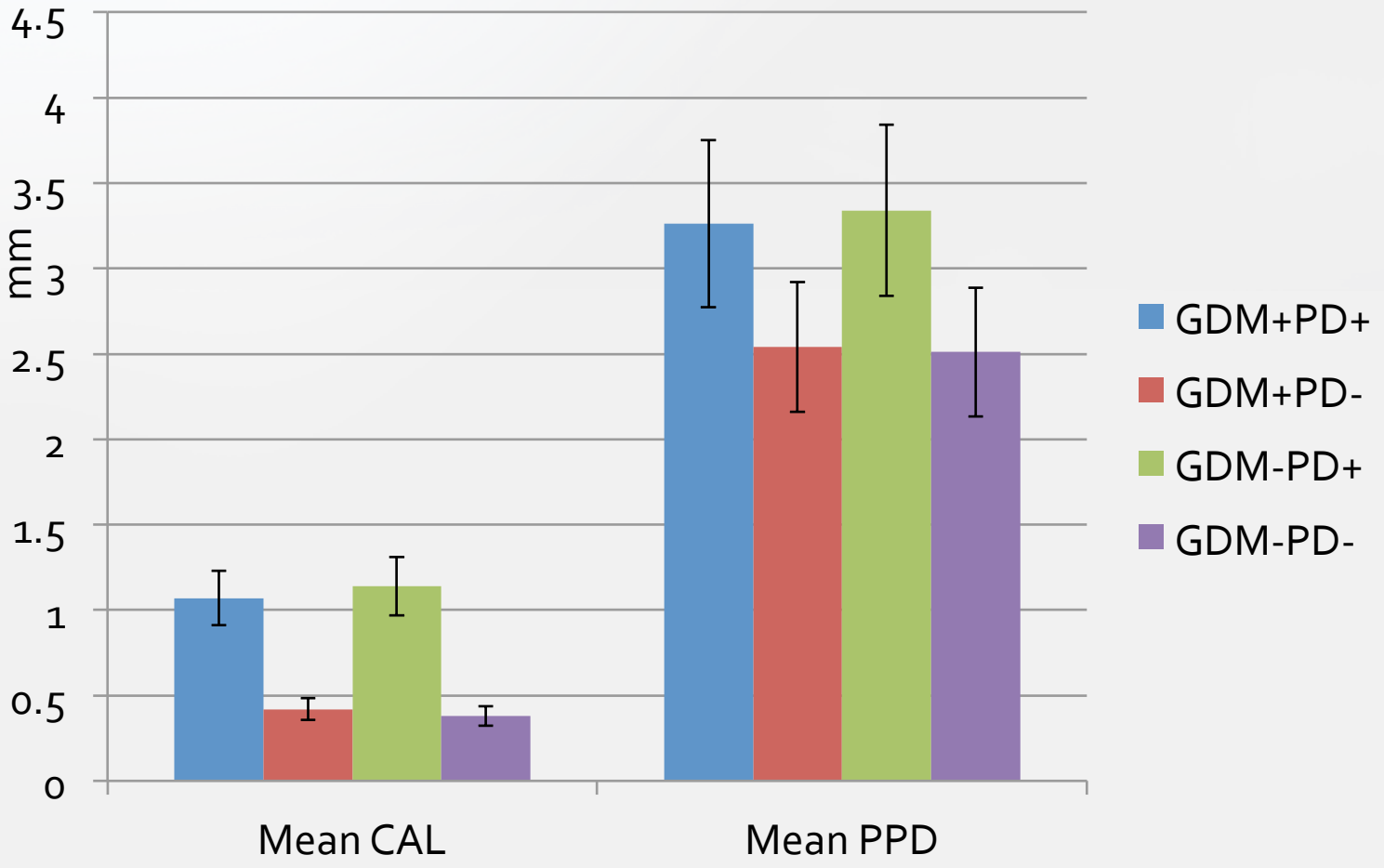
Diagnostics & Alzheimer's Disease

Antibody in AD study population

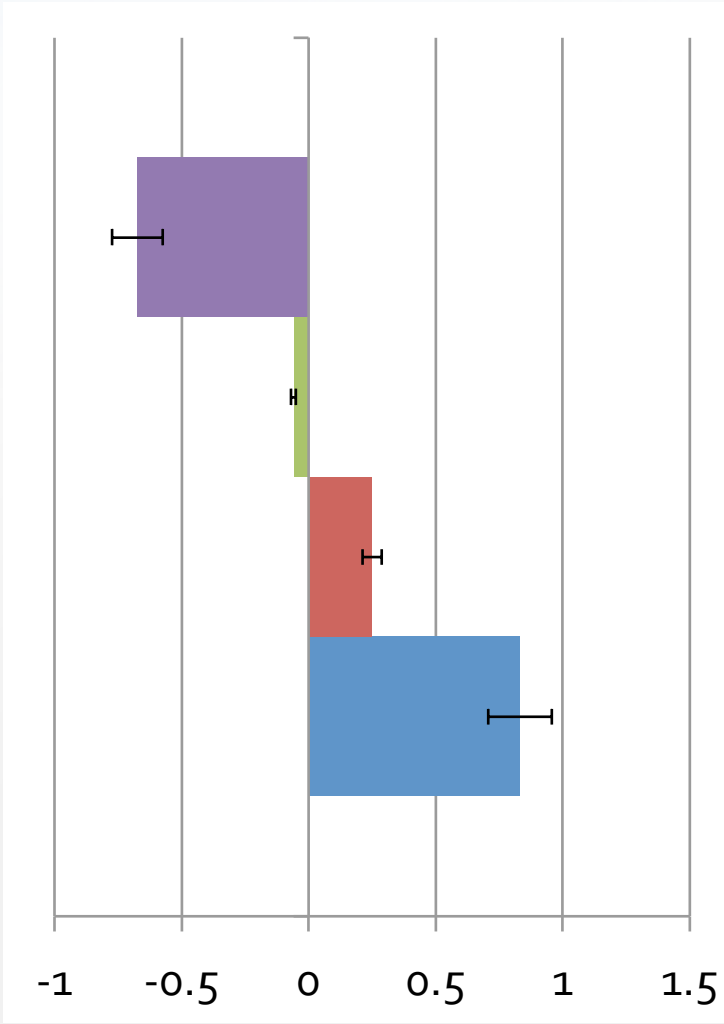


Diagnostics & Gestational Diabetes

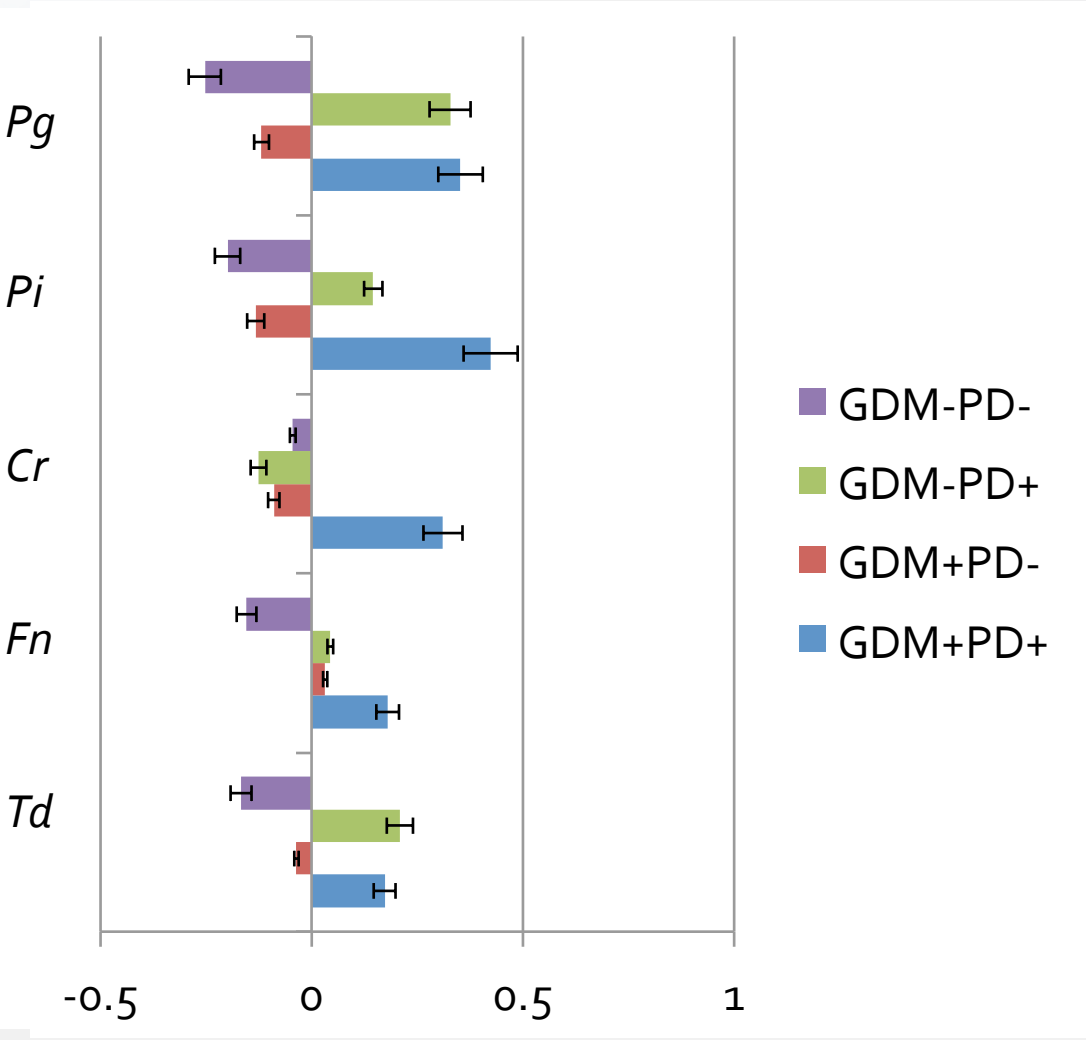
Characteristics of Gestational Diabetes



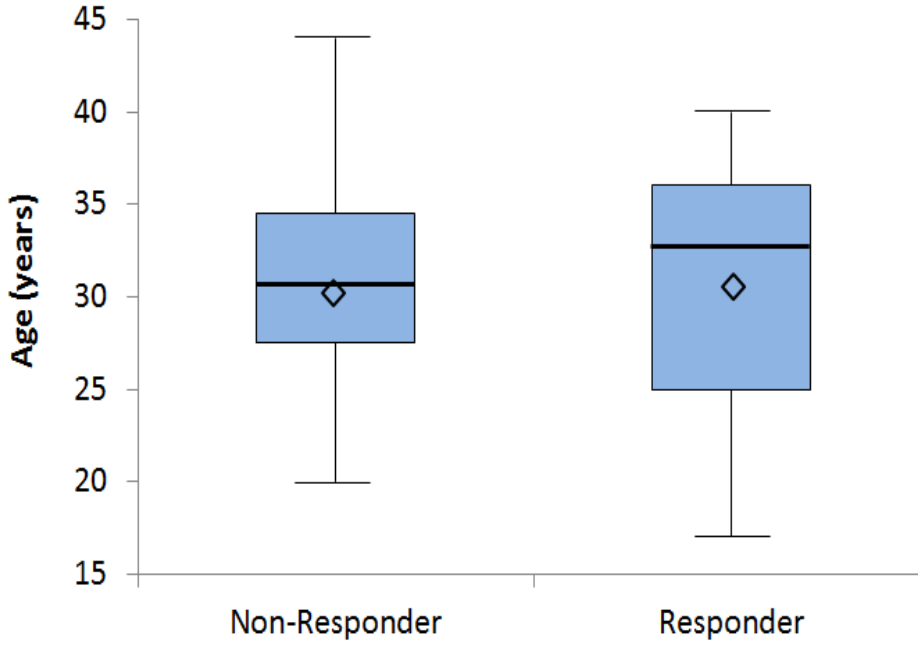
Characteristics of Gestational Diabetes

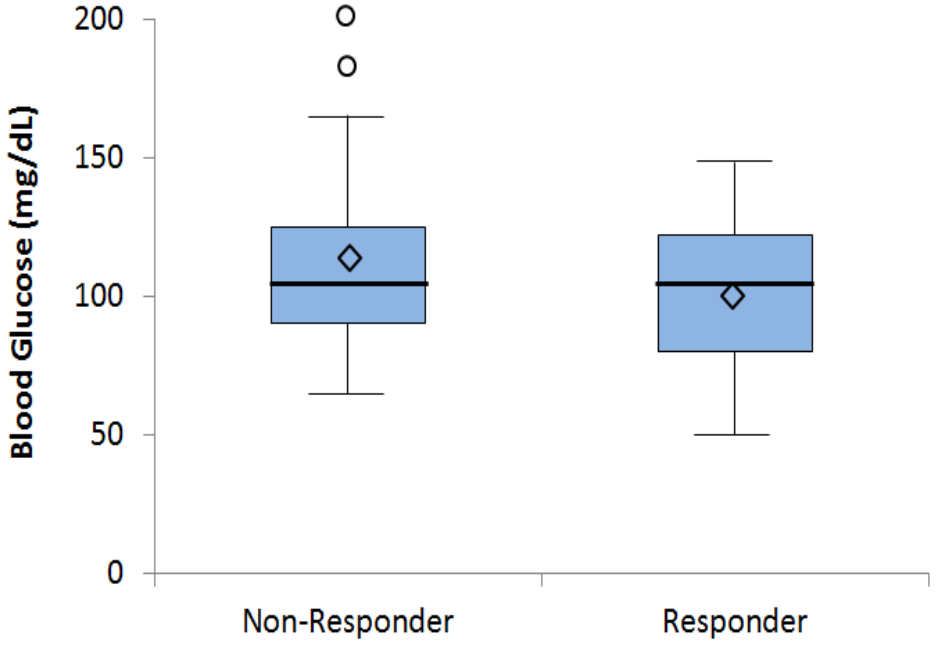


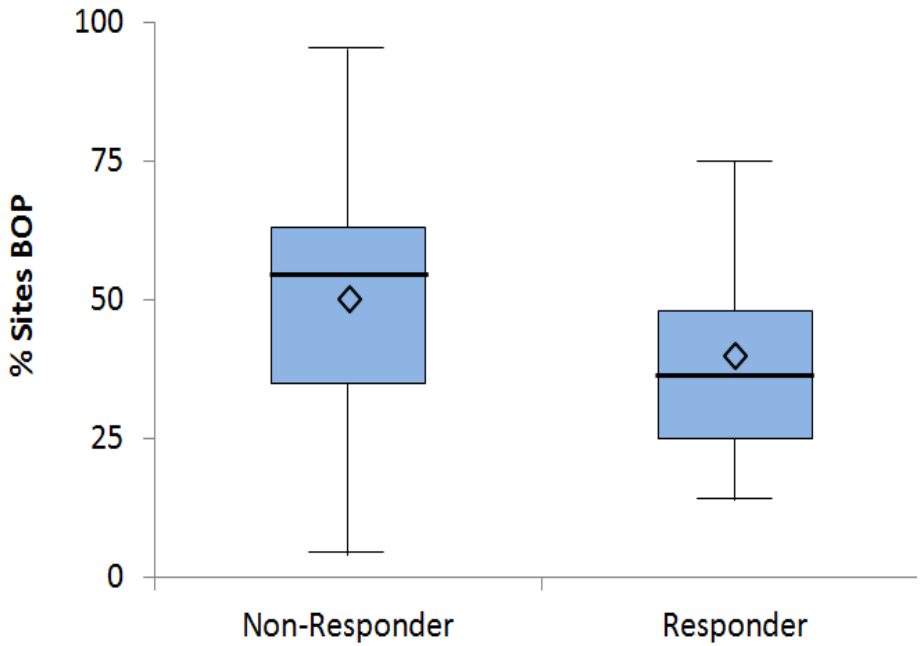
Inflammatory Mediator z-score

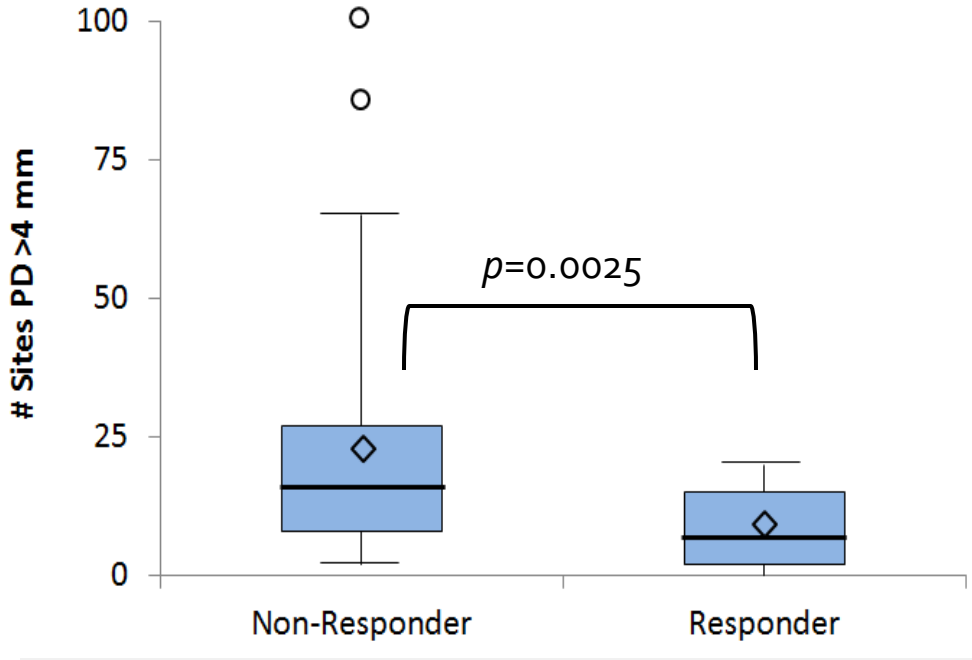


Antibody z-score


Age (years)
45
40
35
30
25
20
15
Non-Responder
Responder


Blood Glucose (mg/dL)
200
150
100
50
0
Non-Responder
Responder

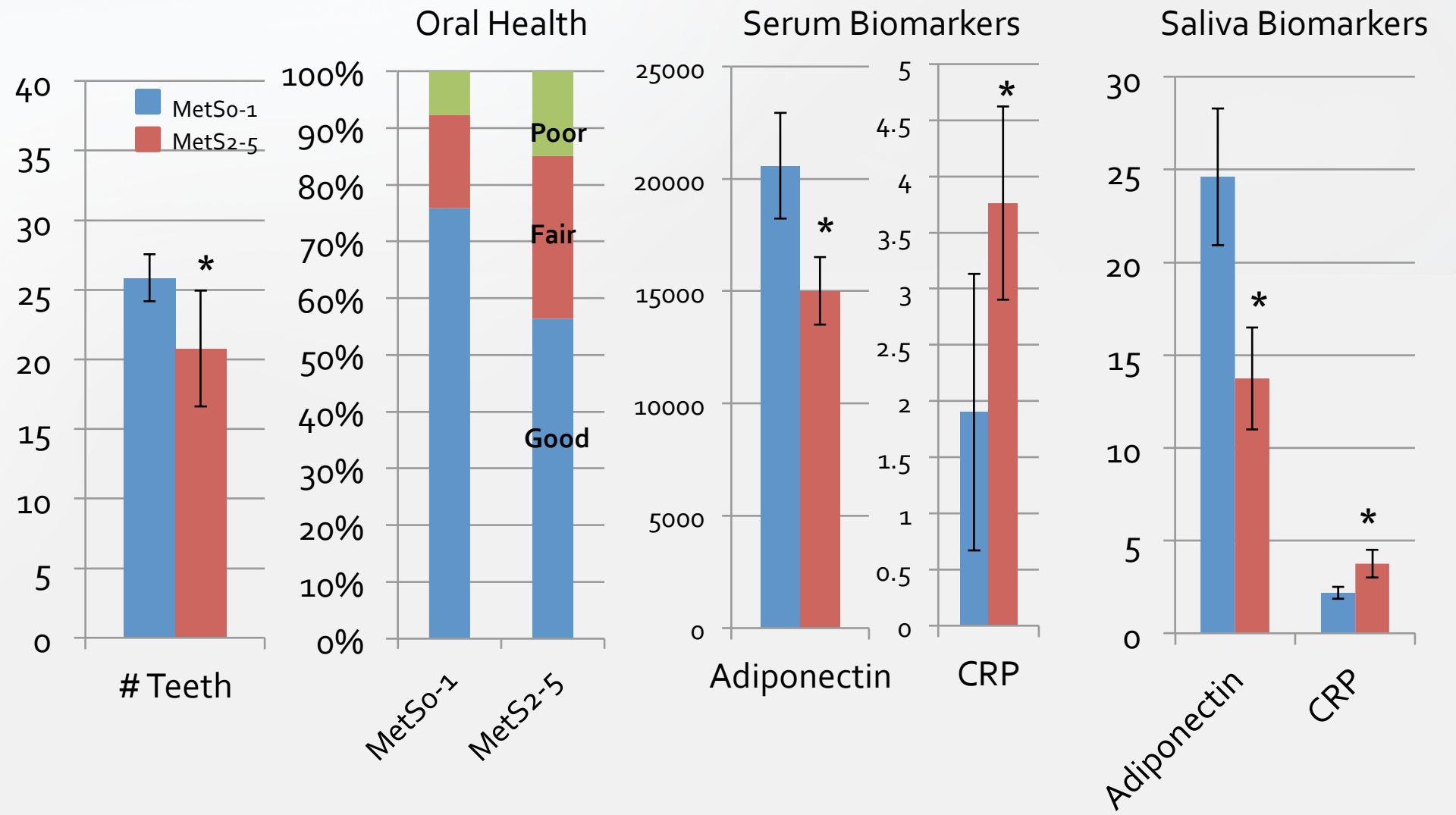

% Sites BOP
100
75
50
25
0
Non-Responder
Responder


Sites PD>4 mm
100
75
50
25
0
p=0.0025
Non-Responder
Responder

Metabolic syndrome (MetS)

- Multifactorial pathophysiologic changes that presage diabetes (**5 Key features**)
  - Large waist circumferences (≥35" – women; ≥40" – men)
  - High triglyceride levels (≥150 mg/dL)
  - Reduced HDL cholesterol (≤40 mg/dL – men; ≤50 mg/dL – women)
  - Increased BP (≥130/85 mm Hg)
  - Elevated fasting blood sugar (≥100 mg/dL)

Clinical parameters & biomarkers in patients: Metabolic Syndrome (MetS)



Environmental Factors & Periodontitis

Exposome (E):
All environmental exposures received by an individual during life

Genome (G):

DNA → RNA→ Proteome → Metabolome

Etiology of chronic diseases is a combination of

Exposome + Genome

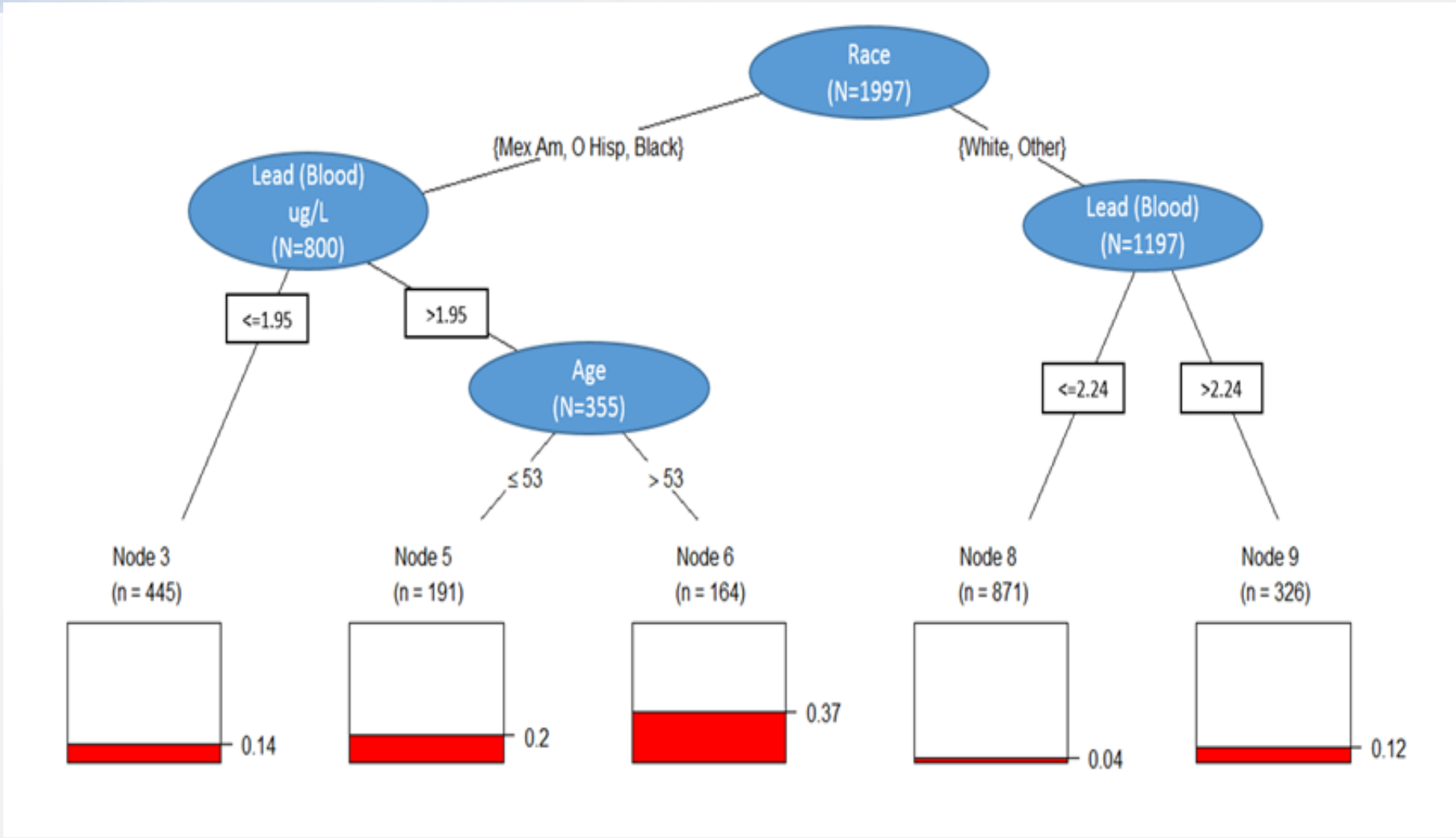
Biomarkers which aim to identify cause, diagnosis, and progression of disease need to represent both dimensions of the **Exposome and Genome**.

Analyses of NHANES 1999-2004:

Out of 156 environmental factors a subset of factors including heavy metals nutrients, PCBs and phthalates are associated with periodontitis

		Smoker		Non-Smoker	
		P-value	OR 95% CI	P-value	OR 95% CI
Lead (ug/dL)	heavy metal	0.0000	1.48 (1.23-1.78)	0.0010	1.34 (1.13-1.57)
cis-b-carotene (ug/	nutrient	0.3280	0.92 (0.79-1.08)	0.0070	0.79 (0.68-0.92)
Retinyl stearate (ug/dL)	nutrient	0.0057	1.31 (1.1-1.57)	0.4295	1.06 (0.91-1.24)
Antimony, urine ng/mL)	heavy metal	0.2570	0.85 (0.65-1.12)	0.0000	1.49 (1.27-1.74)
Mono- cyclohexyl phthalate	phthalates	0.0829	1.34 (0.97-1.84)	0.0096	1.34 (1.09-1.64)
Mono-n- methyl phthalate	phthalates	0.0062	1.49 (1.15-1.93)	0.9290	0.99 (0.75-1.29)
Mono-n-octyl phthalate	phthalates	0.0072	1.55 (1.15-2.1)	0.0632	1.25 (1-1.56)
2,3,7,8-TCDD (fg/g)	dioxins	0.0068	1.76 (1.2-2.58)	0.0693	1.19 (0.99-1.43)
PCB105 (ng/g)	pcb	0.0042	1.55 (1.17-.06)	0.0085	1.34 (1.09-1.65)
PCB146 (ng/g)	pcb	0.0073	1.63 (1.16-.28)	0.3523	1.18 (0.83-1.68)
PCB172 (ng/g)	pcb	0.0017	2.29 (1.42-3.68)	0.0181	1.57 (1.1-2.23)
PCB177 (ng/g)	pcb	0.0025	2.01 (1.32-3.06)	0.0055	1.64 (1.18-2.27)
PCB178 (ng/g)	pcb	0.0018	2.42 (1.45-4.04)	0.0168	1.5 (1.09-2.05)
PCB183 (ng/g)	pcb	0.0003	2.13 (1.47-3.09)	0.0176	1.45 (1.08-1.94)
PCB206 (ng/g)	pcb	0.0006	3.41 (1.86-6.24)	0.0093	1.85 (1.21-2.83)

CART of factors for Periodontitis

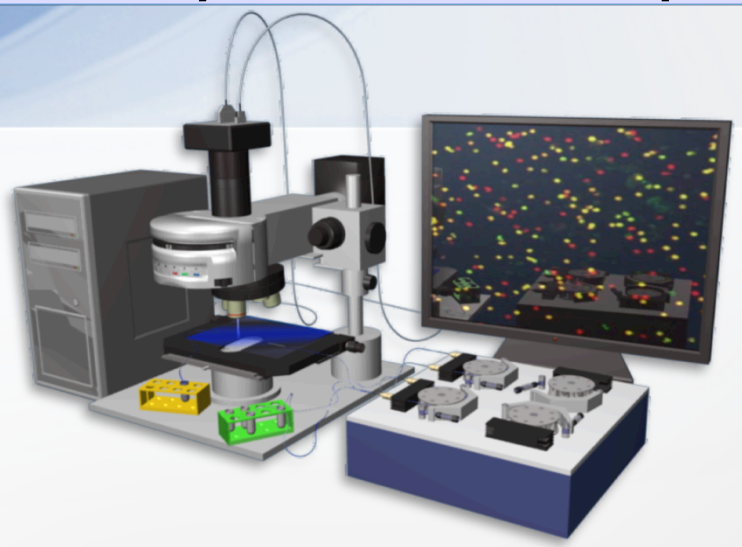


Public Health Opportunities

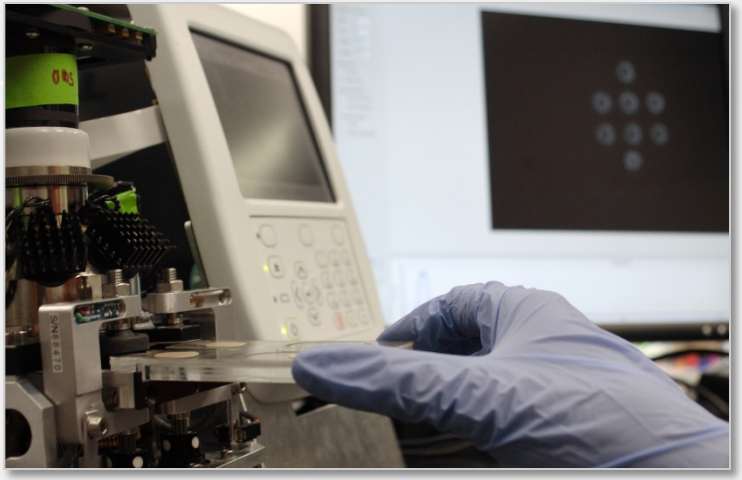
- Point-of-Care (POC) diagnostics
- Diagnostics in non-dental locations
  - Physicians
  - Public health departments
  - FQHC without dental co-located
- Personal OTC diagnostics
- Oral health "Wellness" empowerment
- Oral biomarkers for early systemic disease risk

- Potential for earlier intervention – Lack of a robust treatment toolbox (crux of Precision Healthcare)

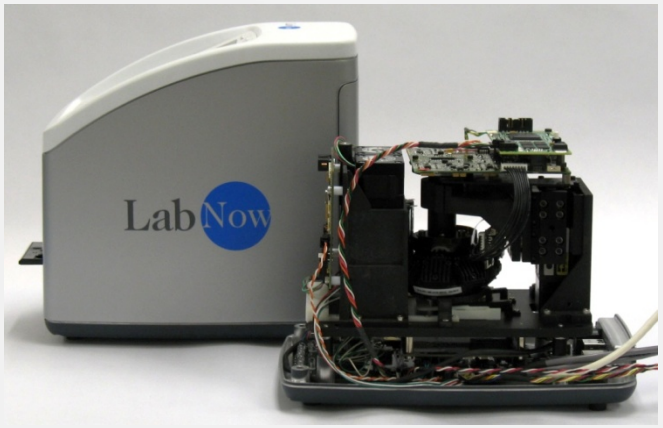
Analyzer Development Stages



Pre-α (lab prototype)



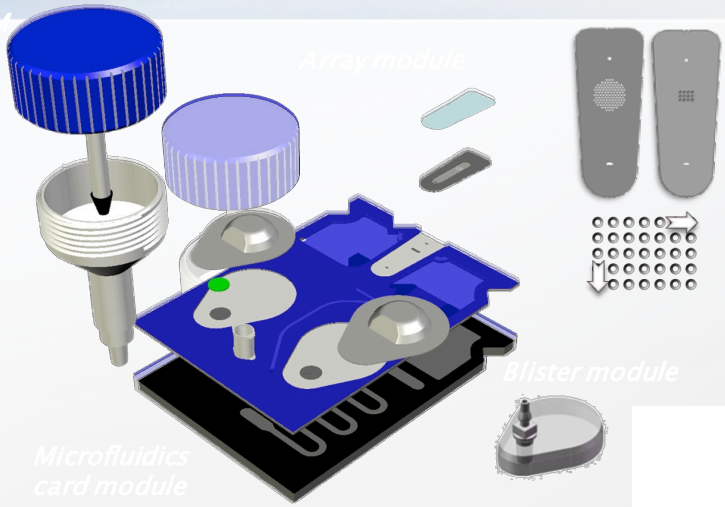
α (open raft, non-form factor)

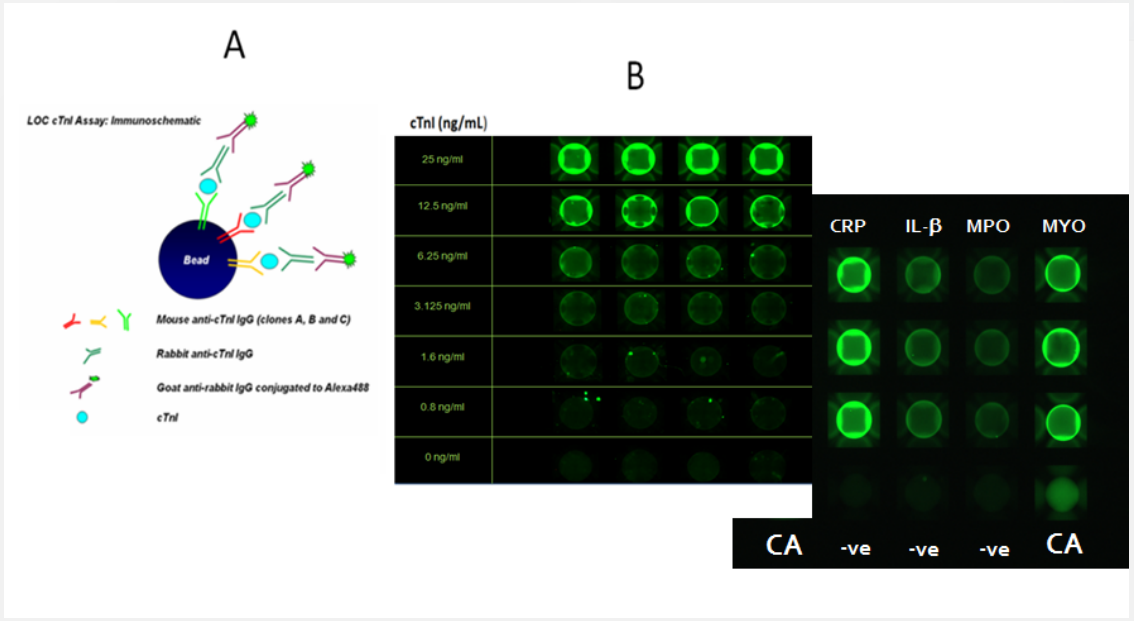


β (form factor)

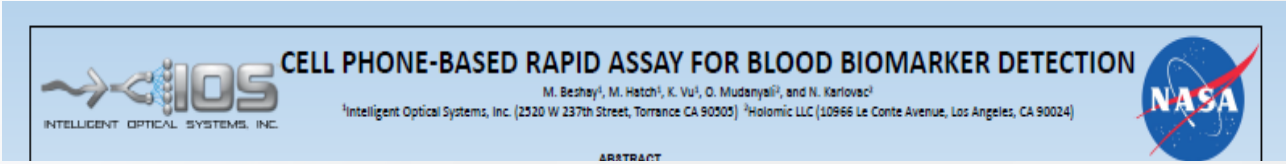
commercial unit (limited supply now)

Nanobiochip Modules

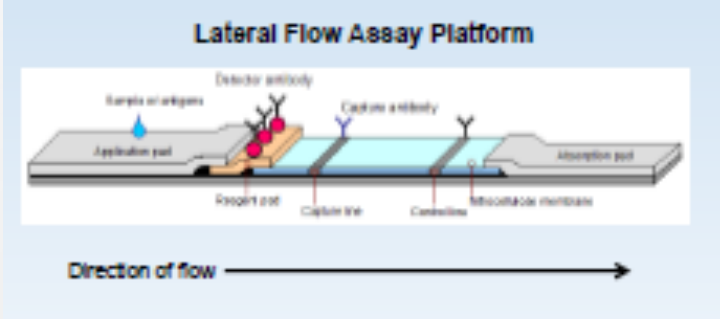




Lateral Flow Chromatography



Salivary MMP-8 in Periodontitis



THE FUTURE!!

"I couldn't repair your brakes, so I made your horn louder." – Steven Wright

- Dental disease is a public health problem
- Dental disease adversely affects individuals, society, and communities
- Dental disease contributes to a long-term economic drain on a community
- Diagnostics for improving early identification and intervention for oral disease in varied venues is necessary
- Develop strategies for linking dental care into general health care through diagnostics for oral-systemic health

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QUESTIONS


SOMETIMES I FEEL THAT I HAVE THE WORST JOB IN THE WORLD!
YA...RIGHT!